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(54) Title: BIOMATERIALS AND BIOSENSORS CONTAINING IMMOBILIZED MODIFIED ANTIBODIES (57) Abstract Biomaterials for use in biomaterial devices, and biosensors, comprise immobilized modified antibodies which are capable of binding antigen or other bioactive species with high affinity and specificity. The modified antibodies are characterized in that at least one thiol-containing moiety is covalently linked to the Fc tail of the antibody so that the antibodies may be immobilized on a substrate with the Fab regions oriented away from the substrate surface.		

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BIOMATERIALS AND BIOSENSORS CONTAINING IMMOBILIZED MODIFIED ANTIBODIES

5 FIELD OF THE INVENTION

This invention relates to modified antibodies, and in particular it relates to antibodies (both monoclonal and polyclonal) which have been modified so that they can be permanently bound or immobilized on a solid substrate in a manner that directs their antigen binding sites away from the surface of the solid substrate.

10

The invention further extends to these modified antibodies immobilized on a solid substrate. The immobilization of the antibodies in a stereospecific orientation such that their antigen binding sites are directed away from the surface of the solid substrate enables the binding of the antibodies to the corresponding
15 specific antigens to be considerably improved. Accordingly, such immobilized modified antibodies are of value in a number of biotechnological and biomedical applications, including biomaterials for use in biomedical devices, and biosensors.

BACKGROUND TO THE INVENTION.

20

In general, biomaterials include materials intended to be used in biomedical devices to be implanted in the body of a human or other animal. The interactions of the biomaterial in a biomedical device with the tissues and fluids of a host body are critical to its biocompatibility and long term stability. Unfavourable interactions lead to processes such as the clotting of blood, inflammation and bacterial
25 infection and may result in unnecessary pain, rejection of the biomedical device and possibly premature death. Thus, the surface of a biomedical device needs to be constructed from a suitable biomaterial, or from a combination of suitable biomaterials, to prevent such adverse interactions.

30

The majority of the biomaterial/host interactions take place at the surface of a biomaterial. The interactions are usually adsorption/desorption processes involving a large variety of proteins, lipids and cells. It is the aim of much biomaterials research to enhance certain favourable interactions whilst

discouraging unfavourable ones. To this end, for example, it is often desirable to attract a particular protein or proteins to the biomaterial on the surface of a biomedical device. The protein or proteins attracted to the surface may enhance the desired biological response while suppressing unwanted consequences. For instance, the selective accumulation on the surface of a biomedical device of adhesive glycoproteins promotes the attachment and growth of a layer of healthy cells. Another example is the selective accumulation on the surface of a cardiovascular device of a protein which can prevent the clotting of blood on the surface and thereby ensure long-term blood compatibility. One or more specific proteins which will exert predictable biomedical responses can be attracted to the surface of a biomedical device with very high selectivity if the surface of the biomedical device is equipped with immobilized antibodies. The immobilized antibodies will bind the desired proteins in a conformation which will enable them to still exert their biological function whereas proteins adsorbed to a synthetic polymer surface (which does not carry immobilized antibodies) typically suffer considerable denaturation. Thus, biomedical devices with surface-immobilized antibodies will provide unprecedented control of the biological events following implantation of the devices.

Biosensors are widely used, particularly in *in vitro* assays, in order to sense or detect the presence of a specific substance. Other uses include use as implantable *in vivo* sensors. One particularly important example of such a biosensor utilizes an immobilized antibody in order to sense or detect the presence in a sample of the specific antigen recognized by the antibody. The molecular sensing of specific solutes in a solution containing a mixture of solutes is important in the fields of medicine, environmental monitoring and the food industry. However, advances in the field of molecular sensing are hampered by nonspecific binding reactions which reduce the sensitivity of the assay. Such undesirable side reactions may be due to low binding affinity of the solute of interest or to high nonspecific binding of the other solutes.

Antibodies provide specific and high affinity binding sites for a large variety of target molecules (antigens) and are therefore ideal candidates to attract

particular proteins to a biosensor or a biomaterial on the surface of a biomedical device. Antibodies are mostly trilobular proteins consisting of an Fc tail and two Fab regions. The Fab regions each contain a binding site for an antigen. In order for the antibody to bind antigen, these binding sites need to be oriented away from the surface. The binding of antigens to antibodies may be sensed by several techniques known in the art, including enzyme-linked immunosorbent assays (ELISAs), surface acoustic waves, surface plasmon polaritons, quartz crystal microbalances, total internal reflection fluorescence, radiolabelled, bioassays, etc.

10

The binding of antibodies to a surface may take place via simple physical adsorption, but the adsorbed antibodies are subject to rapid displacement from the surface by other biological molecules competing for adsorption. Thus, adsorbed antibodies may be replaced very quickly, in particular when the biomaterial is on the surface of a biomedical device which is in contact with a flowing medium, as is the case for instance for the inside surface of a vascular graft. Moreover, a fraction of the antibodies is adsorbed in a way which blocks the antigen binding site. Such random orientation of the antibodies upon adsorption to biomaterial surfaces prevents access of the antigen to all of the antibody binding sites. To obtain optimal antigen density and to give optimal control of antigen orientation (where monoclonal antibodies are being used) requires the maximisation of the antigen-binding activity in the adsorbed antibody layer, and the precisely controlled orientation of the antigen-binding site on the adsorbed antibody as a result of the mechanism of immobilization of the antibody onto the surface. This will not be achieved by non-specific adsorption of the antibodies onto the surface, unless the antibody binding of the surface is sufficiently high as to give orientation due to close packing of the adsorbed antibodies. However a non-specific binding surface with a close packed layer of antibodies will be susceptible to loss of antibody and consequently, disorientation of the remaining layer, following desorption. Moreover, a fraction of antibodies which are adsorbed on a surface would be expected to be denatured; these denatured antibodies will not bind antigen.

30

Each of the techniques described above for sensing the binding of antigens to antibodies require that the antibody be bound to a solid surface. As described above, such binding may take place via simple physical adsorption, but the adsorbed antibody can be washed off with a solvent or can be displaced from the surface in the course of the assay. Moreover, a fraction of the antibodies is adsorbed in ways which block the antigen binding site: if an Fab region of a surface-immobilized antibody is located too close to the surface, steric hindrance and structural distortions impair or prevent antigen binding. Thus, for maximum utilization and detection efficiency the antibody should be oriented such that the binding sites are oriented away from the surface. Random orientation of the antibodies upon adsorption to surfaces prevents access of the antigen to the binding sites of a considerable fraction of the adsorbed antibodies; this reduces the sensitivity of the assay and wastes valuable antibodies.

Desorption of physically adsorbed antibody from a surface may be prevented by immobilization of the antibody via covalent binding rather than by simple adsorption. Permanent covalent binding of the antibody to the surface may be accomplished by a variety of chemical reactions known in the art which covalently couple amine or carboxyl groups on the antibody. For example, amine groups on antibodies may be reacted with carboxyl groups on a surface with the assistance of a water-soluble carbodiimide (International Patent Publication WO 90/05303, Bergstrom *et al.*). However, as amine groups are found on all parts of antibodies, this method does not achieve stereoselectivity for the Fc part; the process orients the antibody randomly and only a fraction of the antibody population is able to bind antigen, thus wasting valuable antibodies. In biomedical device applications, incorrectly oriented antibodies may give rise to an adverse immune response. In addition, in biosensors as described above, the sensitivity of a biosensor assay may be lowered by incorrectly oriented antibodies.

Permanent binding may also be achieved by adsorbing the antibodies onto a polymeric surface and then using a radiofrequency glow discharge plasma in an argon atmosphere to physically crosslink the antibodies to each other and the surface through radical reactions (European patent EP 0 351 950 A2, Hsu *et al.*).

However, such a process would be expected to denature a considerable fraction of the antibodies by the chemically severe and poorly controlled radical reactions.

5 An efficient immobilization strategy would permanently bind the antibodies to the substrate in the correct orientation and under gentle, physiological conditions which would not denature the antibody. One such strategy involves enzymatic cleavage of the Fc tail off the antibody, followed by reduction of interchain disulphide bonds with a thiol-containing compound. The resulting
10 sulfhydryl groups on the antibody may then be attached to a variety of thiol-reactive groups on the surface (Y.Jimbo and M.Saito, *J.Molecular Electronics*, 4 111, 1988). Although this technique is elegant, it is technically very difficult because the disulphide reduction step has to be very carefully controlled so that it does not lead to separation of all the chains in the antibody and to a total loss of binding capacity.

15

A further strategy to orient antibodies on surfaces involves proteins (lectins) which bind to the carbohydrate region on the Fc tail. However, the mitogenicity and immunogenicity of these lectins prohibit many biomedical device applications.

20

Yet another strategy involves the generation of aldehyde groups on the carbohydrate region of the Fc tail of the antibody by treatment with periodate or galactose oxidase. The aldehyde groups of the antibodies are then used for a covalent binding reaction with hydrazide groups on the surface. However, experiments have shown that the resulting surfaces are not optimal: existing

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MicroBIND HZ™ plates produced by Dynatech Laboratories show high nonspecific
~~binding of antibodies which do not contain aldehyde groups, indicating strong~~
adsorption, which is not stereoselective. The lack of specificity of immobilization on these surfaces leads to reduced sensitivity and wastage of the antibodies.

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There is therefore scope for the development of a method which enables the gentle, permanent coupling of oriented antibodies to surfaces while bypassing the problems associated with fragmentation of antibodies or the use of lectins.

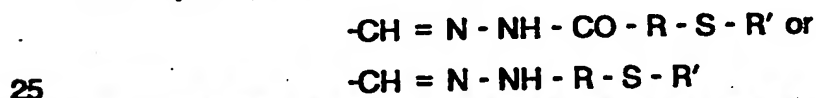
Moreover, any improved method should also reduce the nonspecific binding of antibodies.

SUMMARY OF THE INVENTION.

5 The present invention provides biomaterials for use in biomedical devices, and biosensors, which have attached to at least one surface thereof modified antibodies which are covalently immobilized in such a fashion that stereospecific attachment takes place. The covalent immobilization reaction may involve first the reaction between a thiol-containing hydrazide compound with an aldehyde group
10 in the carbohydrate region of the Fc part of the antibody, and then reaction of the modified antibody with a substrate surface which contains reactive groups capable of forming a covalent bond with the thiolated compound attached to the antibody. In this way the covalently immobilized, modified antibody is gently and permanently attached on to the surface with the Fab parts oriented away from the substrate
15 surface and thus capable of optimal binding with antigen.

Accordingly, in one aspect, the present invention provides a modified antibody, or a fragment or conjugate thereof, which is characterised in that at least one thiol-containing moiety is covalently linked to the Fc tail of the antibody,
20 fragment or conjugate.

Preferably, the or each thiol-containing moiety linked to the Fc tail of the antibody, fragment or conjugate has the formula:



where R' is H or R'; and R and R', which may be the same or different, each represents an aliphatic group, an aromatic or alicyclic ring, an oligo (ethyleneoxide) chain, or a monosaccharide or oligosaccharide group. The group represented by R may have optional substituents for example, one or more halogen or hydroxyl
30 groups, or the like.

The thiol-containing moiety may be linked to the Fc tail by reacting aldehyde groups on the Fc tail with a reagent containing a thiol moiety and a hydrazide,

hydrazine or hydrazone moiety. If necessary, in a first step sugar groups on the Fc tail may be oxidized to produce the aldehyde groups prior to this reaction. In addition, where the thiol-containing moiety produced in this reaction is in the form of a disulphide group, the product is further reacted to liberate a thiol group from the thiol-containing moiety.

The antibody may be monoclonal or polyclonal, and may be derived from a human or other animal. The antibody may be produced by any suitable means including recombinant technology. Antibody fragments and conjugates may also be used, and accordingly references herein to "antibody" are to be understood as including such fragments and conjugates. Antibody conjugates include, for example, conjugates with known reporter or label moieties such as enzymes or radioactive or colorimetric labels.

In a second aspect, this invention provides a substrate having a modified antibody, fragment or conjugate as described above immobilized on a surface thereof in the correct orientation to bind antigen. In order to immobilize the modified antibody, fragment or conjugate on the surface of the substrate, the thiol-containing moiety or moieties linked to the Fc tail thereof are reacted with thiol-reactive groups at the surface of the substrate. Such groups may, for example, be gold atoms deposited on the surface, or other thiol-reactive groups formed on the surface, e.g. surfaces bearing maleimide, sulfhydryl, pyridyldithio or iodoacetyl groups.

The substrate may be, for example, polymeric, metal, ceramic quartz, glass or liquid crystal, or a variety of other suitable substrates known in the art. The substrate is preferably a solid substrate, and may be either porous or non-porous. The substrate may also comprise a thin film or coating of a suitable substrate material on a solid carrier support or base. The support or base may be coated with a thin film which contains thiol-reactive groups, or the substrate may be treated to form thiol-reactive groups on the surface thereof. By way of example, a polymeric film substrate may be exposed to a hydrogen sulfide glow discharge to form sulfhydryl groups on the surface thereof.

Gold is a preferred substrate for biosensor applications, however other suitable substrates for these applications include, for example, polymer, quartz and glass. Preferred substrates for biomedical devices are metals, ceramics and polymers.

5

In a third aspect, this invention provides a biomaterial for use in biomedical devices, or a biosensor, comprising immobilized modified antibodies, fragments or conjugates as described above which are capable of binding antigen with high affinity and specificity. As described above, the substrate for said biomaterial or
10 biosensor may be comprised of a polymer, ceramic, metal or a variety of other suitable substrates known in the art.

In yet a further aspect, this invention provides a biomaterial comprising immobilized modified antibodies, fragments or conjugates as described above
15 which are capable of binding a bioactive species. The bioactive species may comprise proteins, particularly enzymes or other antibodies, and/or cells which form a further layer on the biomaterial. The further layer may, for example, comprise a second antibody layer, a fibronectin layer or a layer of endothelial cells.

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Further features of the present invention will be apparent from the following description of preferred embodiments of the invention and the Examples.

BRIEF DESCRIPTION OF THE FIGURES.

Fig. 1 shows examples of reactions whereby antibodies can be thiolated.

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~~Fig. 2 shows examples of reactions whereby thiolated antibodies may be~~
permanently immobilized on a variety of surfaces.

Fig. 3 shows ELISA results which demonstrate the immobilization of anti-
30 fibronectin antibodies on surfaces containing various species, including gold atoms, and maleimide, iodoacetyl and pyridyldithio groups.

Fig.4 shows binding of unmodified and modified anti-HRP antibodies to a gold substrate.

Fig.5 shows binding of unmodified and modified anti-HSA antibodies to a gold substrate.

DESCRIPTION OF PREFERRED EMBODIMENTS.

The immobilization of antibodies on any surface requires a minimum of two conditions: reactive species on the surface and at least one reactive group on the antibody. In the present invention, thiol groups are attached on to the carbohydrate region of the Fc tail of the antibody according to Fig.1. The aldehyde groups may be generated by any method which oxidizes vicinal diols to aldehydes. For example, treatment of 0.01-100 mg/mL antibody (preferably 5-10 mg/mL) with 0.1-1000 mM sodium periodate (preferably 10-50 mM) at 0-60°C (preferably 4-37°C) for 1-1000 minutes (preferably 10-120 minutes) in the dark. The reaction may be quenched by the addition of 0.1-1000 mM ethylene glycol (preferably 20-100 mM) for 1-1000 minutes (preferably 10-30 minutes). The oxidized antibodies are then separated from the low molecular weight reagents by dialysis or by gel permeation chromatography.

20

The oxidized antibodies may be thiolated using a variety of reagents which contain at least one thiol group and at least one hydrazide, hydrazine or hydrazone group. Examples of useful compounds include 2-acetamido-4-mercaptobutyric acid hydrazide or generally any compound which includes the structures:- $R'-S-R-NHNH_2$ or $R'-S-R-C=NNH_2$ where R' is H or R'' , and R and

saturated alkyl group, an aromatic moiety, an alicyclic ring, an oligo(ethyleneoxide) chain or a monosaccharide or oligosaccharide. R and R'' may optionally contain several halogen atoms, hydroxyl groups and the like as long as the essential thiol and hydrazide remain reactive. Thus 0.01-100 mM thiolating reagent (preferably 1-10 mM) is added to 0.01-100 mg/mL antibody (preferably 1-10 mg/mL) at 0-60°C (preferably 4-37°C) for up to 48 hours (preferably 1-20 hours). The thiolated

30

antibodies are then separated from the low molecular weight reagents by dialysis or by gel permeation chromatography.

These thiolated antibodies are then reacted with surfaces containing various
5 species, including gold atoms, thiols, maleimides, iodoacetyl groups and
pyridyldithio groups (see Fig. 2). A gold surface may be generated by a number
of methods known in the art, such as sputter coating or evaporative techniques
for the deposition of thin gold films onto a variety of carrier materials. Where the
carrier material is glass, the surface is preconditioned with a layer of Cr 0.1-100
10 nm thick (preferably 0.5-10 nm) deposited by thermal evaporation. The said Cr
layer is then overcoated with gold 1-1000 nm thick (preferably 10-200 nm).

Alternatively, the thiolated antibodies may be reacted with thiol groups which
are attached to the surface of a polymeric carrier material. Thiol groups may be
15 formed on polymer surfaces by conventional chemical reactions. For example,
surfaces bearing amine groups (e.g., formed by exposure of the surface to a
radiofrequency glow discharge in an amine-containing vapour) may be treated with
2-iminothiolane or N-acetyl homocysteine lactone in an alkaline buffer.

20 Thiol groups may also be formed on polymers by treatment of the surface
with a radiofrequency glow discharge plasma struck in a mercaptan vapour, e.g.
hydrogen sulphide or an alkyl thiol. The thiol groups on the polymer surface may
then be coupled to the thiolated antibodies in the presence of an oxidant such as
hydrogen peroxide or oxygen.

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~~Maleimide, iodoacetyl and pyridyldithio groups may be formed on surfaces~~
by a variety of techniques, such as the treatment of an amine-containing surface
(e.g., formed by exposure of the surface to a radiofrequency glow discharge in an
amine-containing vapour) with heterobifunctional reagents comprising an N-
30 hydroxysuccinimide, a spacer arm and either a maleimide, iodoacetyl or
pyridyldithio group (Y. Jimbo and M. Saito *J. Molecular Electronics*, 4 111, 1988).
The surfaces to be modified are placed in an aqueous buffer, pH 5-10 (preferably
pH 6-8) containing 0.01-100 mM (preferably 1-10 mM) of the heterobifunctional

reagent for 1-1000 minutes (preferably 10-100 minutes) at 4-70 °C (preferably 20-40 °C).

The following Examples illustrate various features of the present invention.

5

EXAMPLE 1

Monoclonal antibodies to fibronectin were treated with 10 mM sodium periodate in 0.1 M sodium acetate buffer, pH 5.0 for 20 minutes in the dark at 20 °C. The excess periodate was separated from the oxidized antibody by gel permeation chromatography on Sephadex G-25 using 0.1 M sodium acetate buffer, pH 5.0 as the eluent. The oxidized antibody was reacted with 1 mg/mL 2-acetamido-4-mercaptobutyric acid hydrazide for 16 h at 4 °C and then dialysed against 0.1 M sodium acetate buffer, pH 5.0 at 4 °C with 3 changes of buffer. The product contains antibody which is thiolated in the carbohydrate region of the Fc tail.

Glass coverslips (22 mm diameter) were coated with gold using thermal evaporation. The gold-coated coverslips were loaded into enzyme linked immunosorbent assay (ELISA) plates which had been previously treated with 10 mg/mL bovine serum albumin (BSA) for 16 hours at 4°C in order to block nonspecific binding of proteins to the ELISA plates. The sample in each well was covered with a solution of thiolated antibodies (100 µL, 20 µg/mL) and incubated for 16 hours at 4°C and then for a further 1 hour at 37°C. The wells were then rinsed and subjected to an ELISA assay involving fibronectin, biotinylated gelatin

antibodies which are undenatured and are oriented with the antigen binding sites not masked by the surface. The results of the assay (Fig. 3A) show that the thiolated antibody is immobilized on the gold surface.

30

Comparative Example 2

Example 2 is identical to example 1, except that the gold-coated coverslips

were placed into 100 mL of 95% EtOH containing 1 mM mercaptoethylamine hydrochloride and 1 mM triethylamine in order to place amine groups on the surface. After 16 hours of reaction at 20°C, the samples were rinsed with EtOH.

- 5 When the samples were subjected to an ELISA assay (results in Fig. 3B), there was negligible antibody bound, showing that the thiolated antibodies do not bind to gold surfaces which have been overcoated with species which do not react with thiol groups.

10

EXAMPLE 3

- Example 3 is identical to example 2, except that the amine-containing surfaces were placed in 10 mL of phosphate buffered saline (PBS; 50 mM sodium phosphate, 100 mM sodium chloride, pH 7.4) containing 5 mg of
15 sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB). After 150 minutes at 20 °C, the samples were rinsed with PBS.

- When the samples were subjected to an ELISA assay (results in Fig. 3C), there was a substantial amount of antibody bound, showing that the thiolated
20 antibodies may be immobilized on surfaces bearing iodoacetyl groups.

EXAMPLE 4

- Example 4 is identical to example 3, except that sulfosuccinimidyl 4-(p-
25 maleimidophenyl)butyrate (sulfo-SMPB) was used in place of sulfo-SIAB. When the samples were subjected to an ELISA assay (results in Fig. 3D), there was a substantial amount of antibody bound, showing that the thiolated antibodies may be immobilized on surfaces bearing maleimide groups.

30

EXAMPLE 5

- Example 5 is identical to example 3, except that N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) was used in place of sulfo-SIAB. When the

samples were subjected to an ELISA assay (results in Fig. 3E), there was a substantial amount of antibody bound, showing that the thiolated antibodies may be immobilized on surfaces bearing pyridyldithio groups.

5

Comparative Example 6

Polyclonal antibodies to horse radish peroxidase (anti-HRP) were oxidized with 10 mM sodium periodate and then purified by gel permeation chromatography as in Example 1. One μg of oxidized anti-HRP antibodies was placed in contact
10 with a gold-coated coverslip and the deposition of oxidized anti-HRP antibodies onto the gold was monitored by ellipsometry. The results in Fig.4A show that a ca. 10 Å layer formed on the gold.

EXAMPLE 7

15

The oxidised anti-HRP antibodies from comparative example 6 were thiolated with 2-acetamido-4-mercaptopbutyric acid hydrazide as in Example 1. One μg of thiolated anti-HRP antibodies was placed in contact with a gold-coated coverslip and the deposition of oxidized anti-HRP antibodies onto the gold was
20 monitored by ellipsometry. The results in Fig.4B show that a ca. 33 Å layer formed on the gold.

The results in Fig. 4A-B therefore show that thiolation of anti-HRP antibodies increases their binding to gold surfaces.

25

Monoclonal antibodies to human serum albumin (anti-HSA) were oxidized with 10 mM sodium periodate and then purified by gel permeation chromatography
30 as in Example 1. A solution of the oxidized anti-HSA antibodies (250 μL , 70 $\mu\text{g/mL}$) was placed on a gold coated prism and the deposition of oxidized anti-HSA antibodies onto the gold was monitored by surface plasmon resonance. The results in Fig.5A show that the reflectivity changed by ca 8 "units", representing

nonspecific binding of the oxidized antibody onto the gold surface. The antibody solution was removed, the surface washed, and a solution of the antigen (50 μ L, 1 mg/mL) was placed in contact with the antibody which was non-specifically bound to the surface. The results in Fig.5C show that the reflectivity changed by only 1.1 "units", demonstrating that non-specifically bound antibody does not bind antigen very efficiently. The ratio of antigen to antibody binding was 0.13 (see Fig.5E).

EXAMPLE 9

10

The oxidized anti-HSA antibodies from comparative example 8 were thiolated with 2-acetamido-4-mercaptobutyric acid hydrazide as in Example 1. A solution of the thiolated anti-HSA antibodies (250 μ L, 70 μ g/mL) was placed on a gold coated prism and the deposition of thiolated anti-HSA antibodies onto the gold was monitored by surface plasmon resonance. The results in Fig.5B show that the reflectivity changed by ca. 6 "units". The antibody solution was removed, the surface washed, and a solution of the antigen (50 μ L, 1 mg/mL) was placed in contact with the antibody bound to the surface. The results in Fig.5D show that the reflectivity changed by 2.1 "units", demonstrating that thiolated antibodies immobilized on a gold surface bind antigen more efficiently than do non-specifically bound antibody. The ratio of antigen to antibody binding was 0.35 (see Fig.5F).

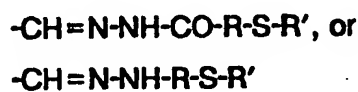
The results in Fig.5A-5 show that although thiolation of anti-HRP antibodies does not increase the amount of antibody bound to gold surfaces, it does increase the amount of antigen bound to the antibody, as well as the ratio of

It will be appreciated that the above detailed description and Examples are included for the purposes of exemplification only, and not by way of limitation of the invention, and that modifications and variations may be made thereto without departing from the ambit of the present invention.

CLAIMS:

1. A modified antibody, or a fragment or conjugate thereof, which is characterized in that at least one thiol-containing moiety is covalently linked to the Fc tail of the antibody, fragment or conjugate.

2. A modified antibody according to claim 1, wherein the or each thiol-containing moiety linked to the Fc tail of the antibody, fragment or conjugate has the formula:



wherein R' is H or R"; and R and R", which may be the same or different, each represents an optionally substituted aliphatic group, aromatic or alicyclic ring, oligo(ethyleneoxide) chain, or monosaccharide or oligosaccharide group.

3. A modified antibody according to claim 2 wherein the optional substituents on the group, ring or chain represented by R and R" comprise one or more halogen or hydroxyl groups.

4. A modified antibody according to claim 1, 2 or 3, which is a monoclonal antibody derived from a human or other animal, or a fragment or conjugate thereof.

5. A modified antibody according to claim 1, 2 or 3, which is a polyclonal antibody derived from a human or other animal, or a fragment or conjugate thereof.

6. A method for the preparation of a modified antibody, fragment or conjugate according to claim 1, which comprises the steps of:

- (i) if necessary, oxidizing sugar groups on the Fc tail of the antibody to produce aldehyde groups; and

- (ii) reacting said aldehyde groups on the Fc tail with a reagent containing a thiol moiety and a hydrazide, hydrazine or hydrazone moiety.
7. A method according to claim 6, further comprising the step of liberating a thiol group from the thiol-containing moiety.
8. A method according to claim 6, wherein said oxidation of sugar groups on the Fc tail is carried out by treatment with sodium or other periodate.
9. A method according to claim 6, 7 or 8, wherein said aldehyde groups on the Fc tail are reacted with a compound of the formula:
- $$\text{R}'\text{-S-R-NHNH}_2, \text{ or}$$
- $$\text{R}'\text{-S-R-C=NNH}_2,$$
- wherein R and R' are as defined in claim 2.
10. A method according to claim 9 wherein said compound is 2-acetamido-4-mercaptobutyric acid hydrazide.
11. A substrate having a modified antibody, fragment or conjugate according to any one of claims 1 to 5 immobilized on a surface thereof in the correct orientation to bind antigen.
12. A substrate according to claim 11, which is a solid substrate, which may be porous or non-porous.
-
13. A substrate according to claim 10, which is a polymer, metal, ceramic, quartz, glass or liquid crystal substrate.
14. A substrate according to claim 11, 12 or 13 which comprises a thin film or coating of a substrate material on a solid carrier support or base.

15. A method for the preparation of a substrate according to claim 11, which comprises reaction of the thiol-containing moiety or moieties of said modified antibody, fragment or conjugate with thiol-reactive groups at said surface of the substrate.
16. A biomaterial for use in biomedical devices comprising immobilized modified antibodies, fragments or conjugates according to any one of claims 1 to 5 which are capable of binding antigen with high affinity and specificity.
17. A biomaterial according to claim 13, wherein said modified antibodies, fragments or conjugates are immobilized on a polymer, metal or ceramic substrate.
18. A biomedical device wherein the or a surface thereof comprises a biomaterial according to claim 13.
19. A biosensor comprising immobilized modified antibodies, fragments or conjugates according to any one of claims 1 to 5 which are capable of binding antigen with high affinity and specificity.
20. A biosensor according to claim 19, wherein said modified antibodies, fragments or conjugates are immobilized on a polymer, metal, quartz, ceramic or glass substrate.
21. A biosensor according to claim 20 wherein said substrate comprises a gold
-
22. A biomaterial for use in biomedical devices comprising immobilized modified antibodies, fragments or conjugates according to any one of claims 1 to 5 which are capable of binding a bioactive species.
23. A biomaterial according to claim 22, wherein said immobilized modified antibodies, fragments or conjugates are capable of binding proteins, particularly

- 18 -

enzymes or other antibodies, and/or cells which form a further layer on the biomaterial.

24. A biomedical device wherein the or a surface thereof comprises a biomaterial according to claim 22.

1/5

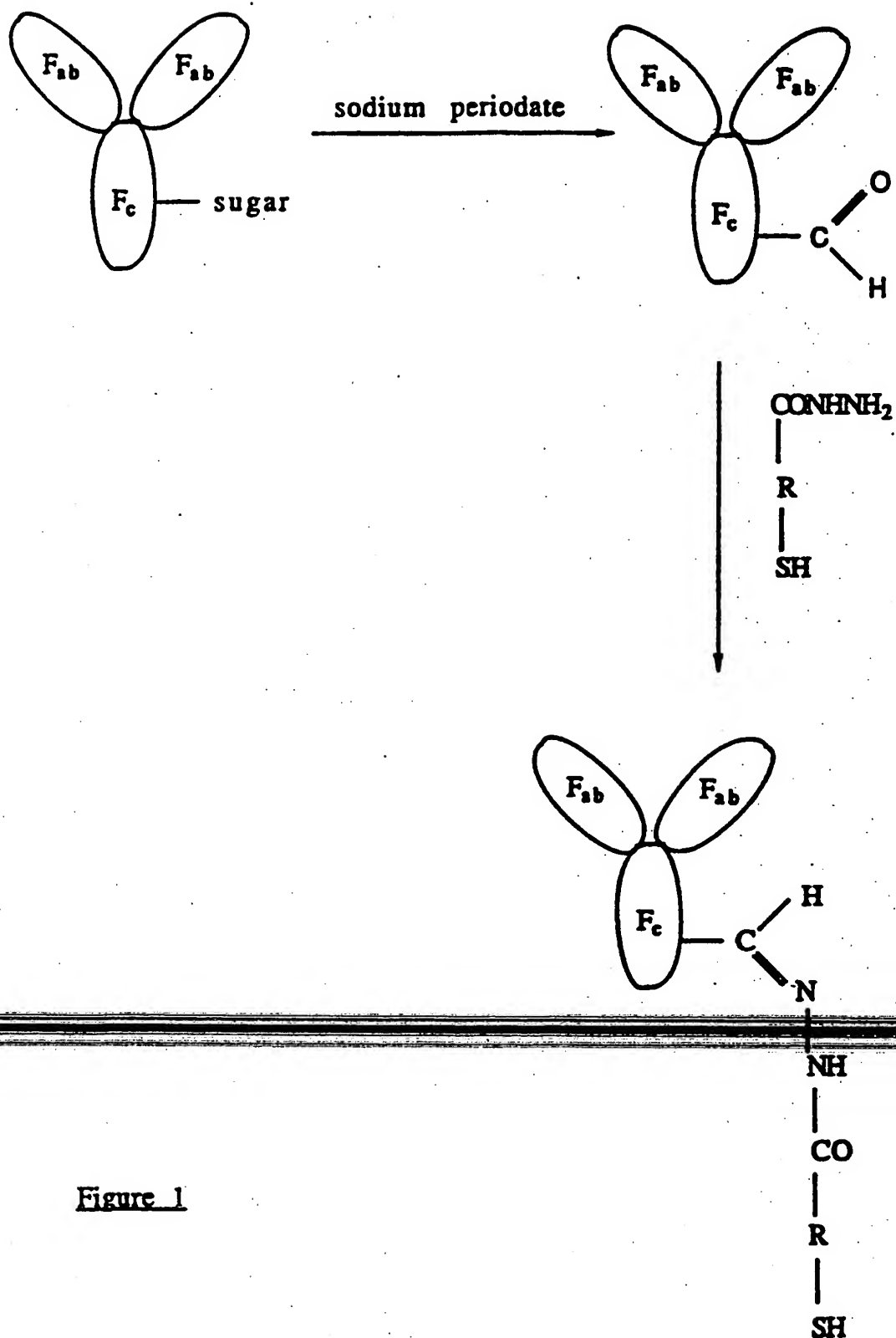


Figure 1

2/5

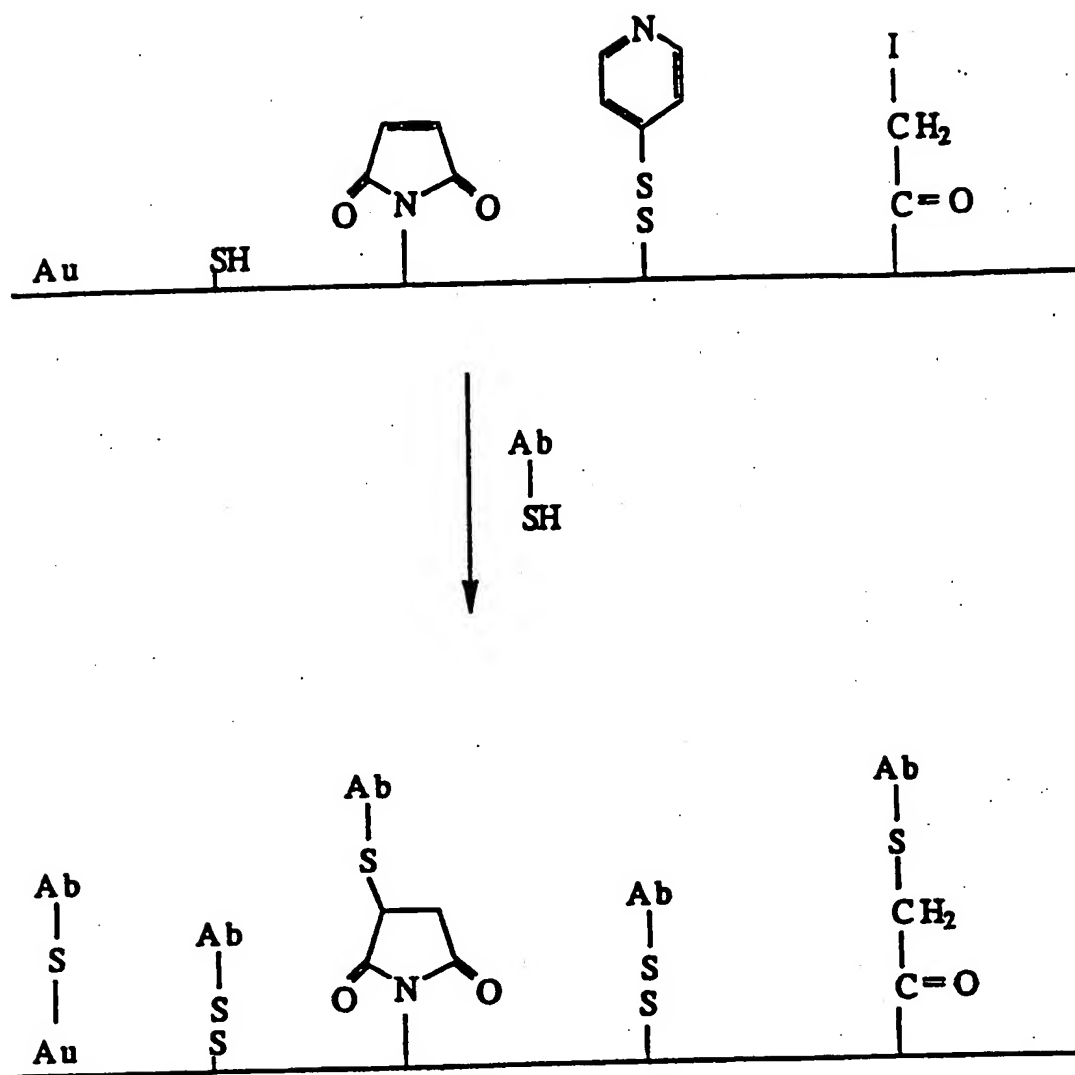
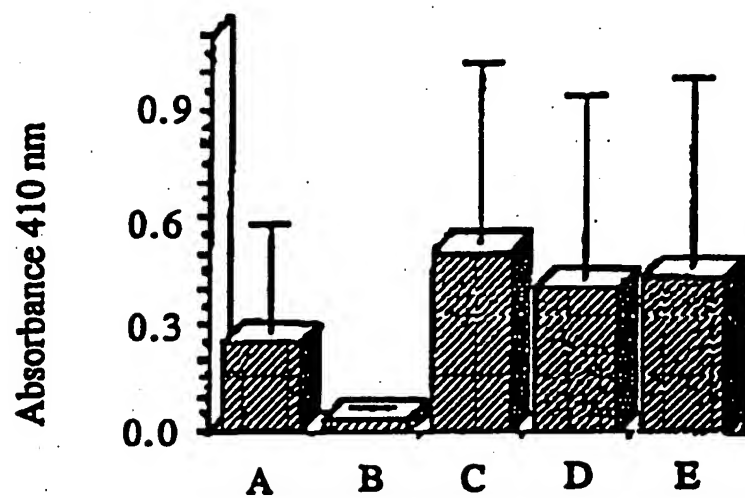


Figure 2

3/5

**Figure 3**

4/5

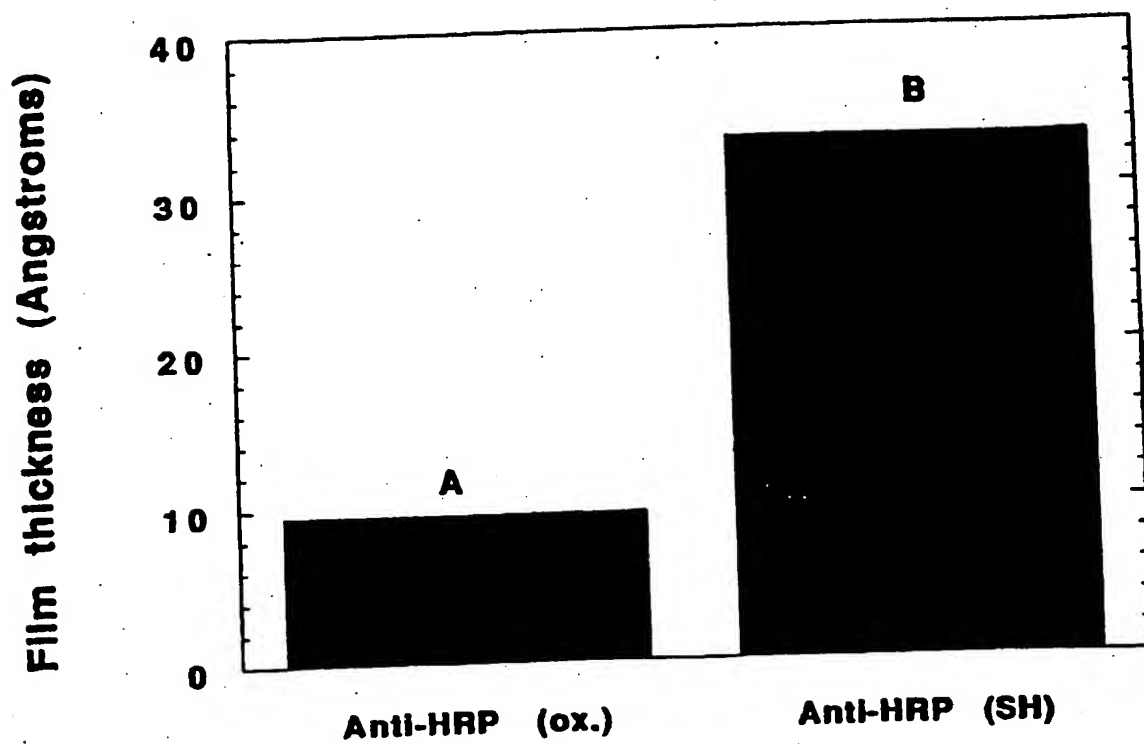


Figure 4

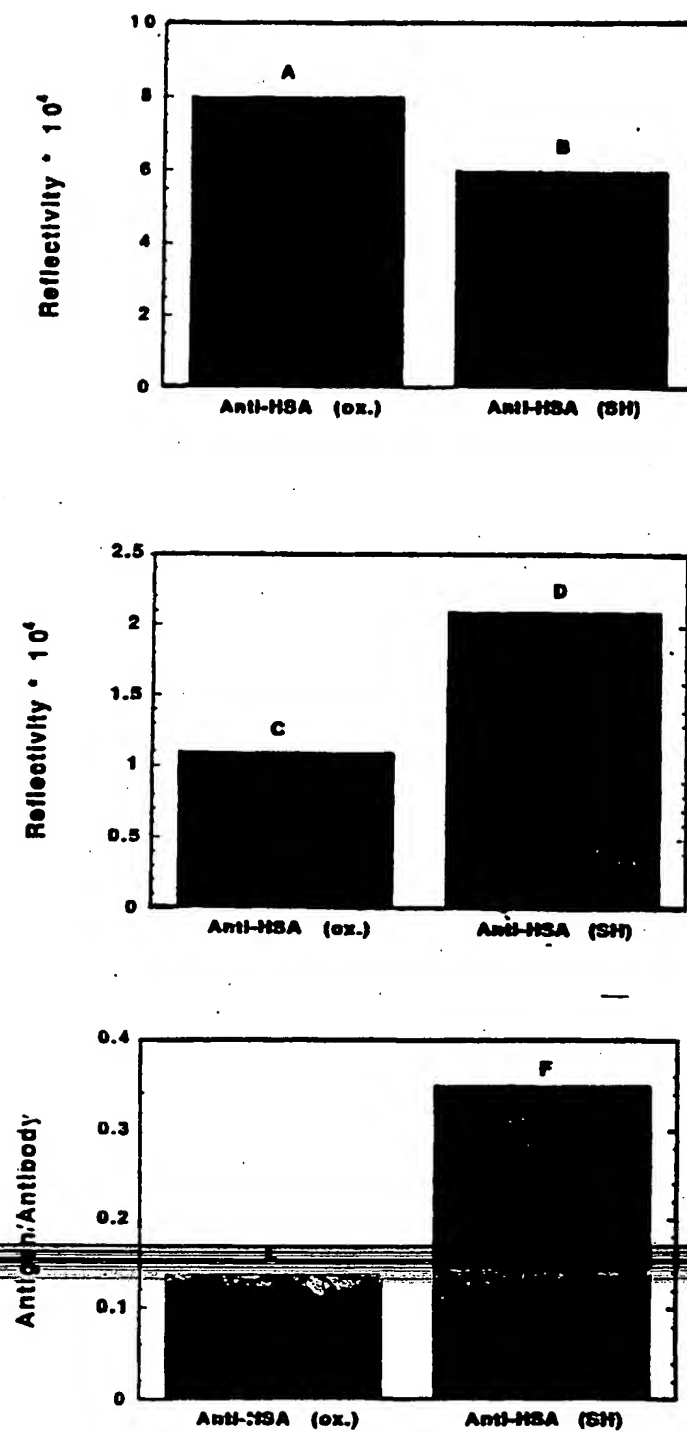


Figure 5

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 93/00386

A. CLASSIFICATION OF SUBJECT MATTERInt. Cl.⁵ C07K 15/12, C07K 15/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07K 15/12, C07K 15/28

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

FILE CAS M: Keywords: ANTIBODY, THIOL

FILE WPAT: Keywords: as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Anal. Chim. Acta, volume 197 issued 1987, Boitieux, J.L., et. al. Reversible Immobilization of An Antibody With a Thio-Substituted Sorbent, pages 229-237.	1,4,5,6 11-24
X	Prot Eng, vol 3, no 8 issued 1990, Lyons, Alan et. al., Site-specific attachment to recombinant antibodies via introduced surface cysteine residues, pages 703-708.	1,4,5,6 11-20,22-24
X	Kato, Kanefusa, Methods in Enzymology, Vol 92, published 1983 by Academic Press, Inc. see pages 345-359	1,4-8,11-20 22-24

Further documents are listed
in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

9 November 1993 (09.11.93)

Date of mailing of the international search report

10 NOV 1993 (10.11.93)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 93/00386

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X	Anal Biochem, Vol. 178, published 1989. Bhatia, Suresh K. et. al. Use of Thiol-Terminal Silanes and Heterobifunctional Crosslinkers for Immobilization of Antibodies on Silica Surfaces, pages 408-413	1,4-8,11-20 22-24